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Echinococcus granulosus: The Distribution of Hydatid Fluid Antigens in the Tissues of the Larval Stage

II. Localization of the Thermostable Lipoprotein of Parasitic Origin (Antigen B)

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YARZABAL, L. A., DUPAS, H., BOUT, D., NAQUIRA, F., AND CAPRON, A. 1977. *Echinococcus granulosus:* The distribution of hydatid fluid antigens in the tissues of the larval stage. II. Localization of the thermostable lipoprotein of parasitic origin (antigen B). *Experimental Parasitology* 42, 115–120. By means of a rabbit monospecific antiserum, the localization of hydatid fluid antigen B, a thermostable lipoprotein was demonstrated in the larval tissues of *Echinococcus granulosus.* The antigen was present in the cuticular "membrane," in the tegument of the protoscoleces, and in the substance contained inside the brood capsules. This distribution corresponds exactly to that of PAS-positive substances described previously in the hydatid cyst of *E. granulosus.* The localization of antigen B and the fact that it was not detected in the excretory system suggest that the subtegumental cells participate in its secretion.

INDEX DESCRIPTORS: *Echinococcus granulosus;* Cestoda; Helminth parasites; Immunology; Antigenicity; Antigen localization; Immunofluorescence test; Hydatidosis; Hydatid fluid; Protoscoleces; *Fasciola hepatica; Schistosoma mansoni; Taenia saginata;* Horse antigens; Rabbit immunization; Antigen B = thermostable lipoprotein.

INTRODUCTION

In the last 10 years two of the soluble antigens of parasitic origin, which are found in the fluid of hydatid cysts of *Echinococcus granulosus,* have been purified and partially characterized. The first, termed antigen "5" by Capron, Vernes, and Biguet (1967), is a highly immunogenic substance which migrates toward the anode in electrophoresis at pH 8.2. Bout, Fruit, and Capron (1974) purified this antigen by immunoadsorption and showed that it was a thermolabile lipoprotein which precipitates in acid medium and possesses both alpha and beta carboxyl esterase enzymatic activities. Yarzabal, Dupas, Bout, and Capron (1976) demonstrated that antigen "5" is localized in the internal layer of the germinal membrane and the parenchyma of the protoscoleces of horse hydatid cysts. They postulated that it was synthesized in specialized cells of the protoscoleces and was then transported by the excretory system to the hydatid cyst cavity.

The second parasitic antigen was described by Oriol, Williams, Perez-Esandi, and Oriol (1971) and was designated antigen B. According to the authors, antigen B is also a substance of lipoproteic nature but is remarkably thermostable and resists temperatures of 100 C for 15 min without great alteration of its antigenicity. It exists

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naturally in various stages of polymerization and stains weakly with Schiff reagent. Strongly positive results were obtained in cutaneous tests for immediate hypersensitivity with this antigen. Oriol and Oriol (1975) have purified the antigen and showed that it is a lipoprotein with a molecular weight equal to 120,000 with optical properties which suggest an organized structure containing about 50% of α-helix.

Williams (1972), while analyzing the thermostable extract of boiled hydatid fluid proposed by Bacigalupo (1925) for the Casoni intradermal test, observed that the reagent prepared by this method contained antigen B and another thermostable substance immunologically identical to a host protein. Bout, Dessaint, Dupas, Yarzabal, and Capron (manuscript in preparation) demonstrated that antigen B is capable of fixing, *in vitro,* IgE-type antibodies present in the serum of patients with hydatidosis.

The object of this work was to determine the distribution of antigen B in tissues of the hydatid cyst by means of immunofluorescence microscopy. Rabbit antiserum against boiled hydatid fluid, made monospecific for antigen B through immunoadsorption, was used for this test.

Material and Methods

Antigens

Whole hydatid fluid (WHF). WHF of *Echinococcus granulosus* was extracted aseptically from healthy hydatid cysts in horses sacrificed at the slaughterhouse in Lille. After centrifugation and dialysis, the hydatid fluid was lyophilized and analyzed immunoelectrophoretically by means of reference antisera to determine its qualitative composition (Capron, Vernes, and Biguet 1967).

Boiled hydatid fluid (BHF). This was prepared according to Williams (1972) by boiling crude hydatid fluid for 15 min and removing the resulting precipitate by means of centrifugation at 50,000g for 30 min. The supernatant was dialyzed against phosphate buffered saline (PBS) at pH 7.2 for 24 hr and lyophilized.

Hydatid cyst sections. Hydatid cysts of equine origin were treated as described in our previous work (Yarzabal, Dupas, Bout, and Capron 1976). After washing three times in PBS, cyst membranes and hydatid "sand" were fixed in "Bouin-Hollande sublimé" solution (HCHO, 2.5 ml; $HgCl_2$, 0.7 g; $(CH_3COO)_2Cu$, 2.0 g; $(NO)_2C_6H_2OH$, 3.3 g; and H_2O, 100.0 ml), dehydrated in alcohol baths at increasing concentrations, treated with butyl alcohol, and embedded in paraffin. The blocks were cut in 5- to 6-μm thick sections and fixed on microscope slides.

Soluble extracts of other helminths. Lyophilized soluble extracts of *Fasciola hepatica, Schistosoma mansoni,* and *Taenia saginata* were prepared according to the method described by Capron, Biguet, Vernes, and Afchain (1968).

Host antigens. Soluble horse liver extract and normal horse serum were lyophilized and used as sources of host antigens.

Immune sera. Antisera were prepared in rabbits against each of the soluble antigens mentioned above, following the protocol described by Capron, Vernes, and Biguet (1967). Monospecific antiserum against antigen B was obtained by absorption of the anti-BHF serum with heterologous soluble antigens (*F. hepatica, S. mansoni, T. saginata,* horse liver, and normal horse serum). The absorption was carried out by mixing 20 mg of each of the helminthic antigens and 10 mg of each of the host antigens with 1 ml of the anti-BHF serum. After incubating for 2 hr at 37 C the mixture was kept at 4 C for 16 hr. At the end of this incubation period the precipitate was separated by centrifugation and the supernatant was used as absorbed serum.

Techniques

Immunoprecipitation tests. Agar double diffusion (Ouchterlony 1948) and immuno-

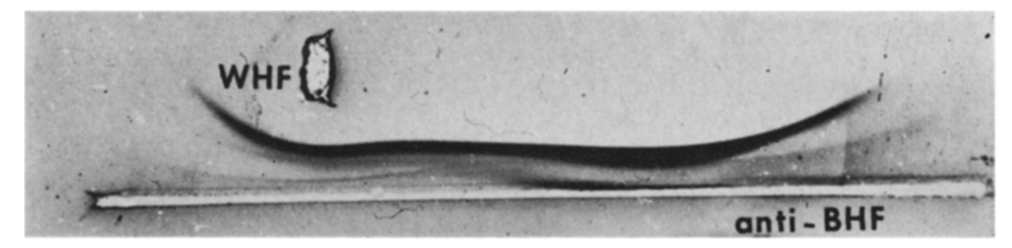


FIG. 1. Immunoelectrophoretic analysis of anti-boiled hydatid fluid serum versus whole hydatid fluid showing antibodies against four *Echinococcus granulosus* antigens. Anode to the right. WHF, whole hydatid fluid; anti-BHF, anti-boiled hydatid fluid.

electrophoresis (Grabar and Williams 1953) were employed. Immunoelectrophoresis was carried out using a support medium of agarose at 0.9% in veronal buffer, 0.05 M, pH 8.2, and a potential gradient of 3 V/cm^{-1} was applied for 3 hr.

Indirect immunofluorescence. The sections of hydatid tissues were deparaffinized and washed in PBS, pH 7.2, and incubated for 30 min at 37 C with the monospecific antiserum against antigen B, diluted 1/40 in PBS. After this incubation, the slides were washed in PBS, pH 7.2, and then incubated for 30 min at 37 C with sheep antirabbit immunoglobulin fluorescent conjugate (commercial preparation, Pasteur Institute, Paris) diluted 1/50. Since nonspecific fixation of this reagent occurred on sections of *E. granulosus* cysts, the conjugate was absorbed with WHF at a concentration of 10 mg/ml. After incubation with the absorbed fluorescent conjugate, the slides were washed three times in PBS and counterstained with Evans' Blue. As a control for specificity, an inhibition reaction was performed by absorbing the monospecific antiserum with the homologous antigen. Fluorescent areas were identified in all cases by staining the slides with hematoxylin and eosin and examining them with a light microscope.

RESULTS

Immunological Analysis of Anti-BHF Antiserum

Double diffusion and immunoelectrophoretic analysis revealed that anti-BHF contained precipitating antibodies to four of the WHF antigens of *Echinococcus granulosus* (Fig. 1). It was shown that they corresponded to (i) the antigen B, (ii) a substance immunologically identical to a host protein, and (iii) two antigens common to *Fasciola hepatica, Schistosoma mansoni,* and *Taenia saginata.*

Absorption of the antiserum with soluble extracts of *F. hepatica, S. mansoni,* and *T. saginata* and with the host antigens elimi-

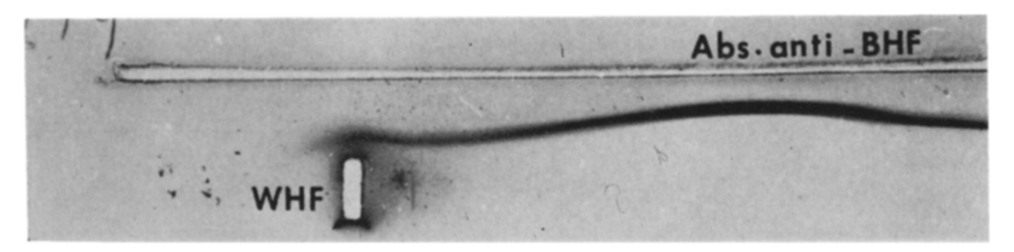


FIG. 2. Immunoelectrophoretic pattern of anti-boiled hydatid fluid absorbed by *Fasciola hepatica, Schistosoma mansoni, Taenia saginata,* horse liver, and horse serum lyophilized extracts. Anode to the right. WHF, whole hydatid fluid; Abs anti-BHF, anti-boiled hydatid fluid serum absorbed by *F. hepatica, S. mansoni, T. saginata,* and host proteins.

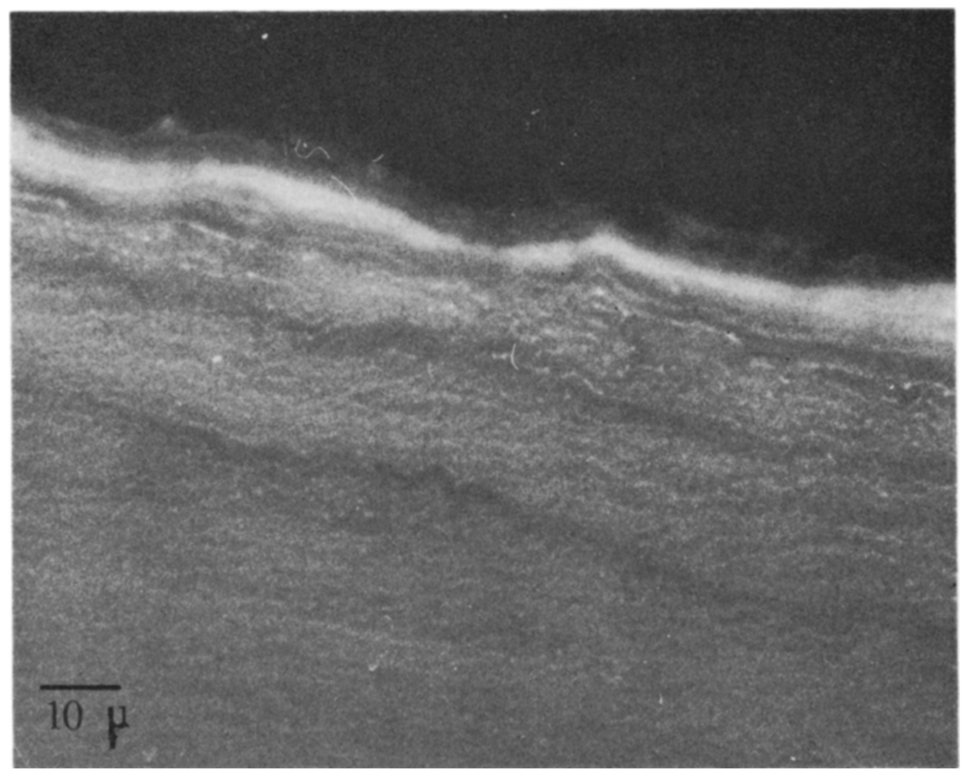


FIG. 3. Section of *Echinococcus granulosus* hydatid "membranes" showing the distribution of antibodies against antigen B (×1000).

nated all reactions against antigens other than antigen B (Fig. 2).

Distribution of Antigen B

In immunofluorescent tests, immunoglobulins in monospecific antiserum against antigen B were fixed at the level of the cuticular "membrane," on the tegument of protoscoleces, and in the interstitial substance of brood capsules (Figs. 3 and 4). The germinal membrane, the wall of the brood capsules, the parenchyma of protoscoleces, and the excretory system did not react with monospecific antiserum.

DISCUSSION

It is clear from these results that the *Echinococcus granulosus* hydatid fluid contains various thermostable substances in addition to antigen B identified by Oriol, Williams, Perez-Esandi, and Oriol (1971). Among them we have confirmed the presence of the antigen immunologically identical to a host protein, as shown by Williams in 1972, but we have also demonstrated two other thermostable antigens which cross-reacted with components of the antigenic mosaic of *F. hepatica, S. mansoni,* and *T. saginata.* It is possible that these common antigens are responsible for cross-reactivity in diagnostic tests experienced by Yarzabal, Schantz, and Lopez-Lemez (1975) and by Schantz, Ortiz-Valqui, and Lumbreras (1975) in patients with neoplasias and parasitic diseases other than hydatidosis.

Indirect immunofluorescence with monospecific anti-B serum revealed that antigen B was distributed in the cuticular membrane, the tegument of the protoscoleces, and the interstitial substance of the brood capsules. This distribution is totally differ-

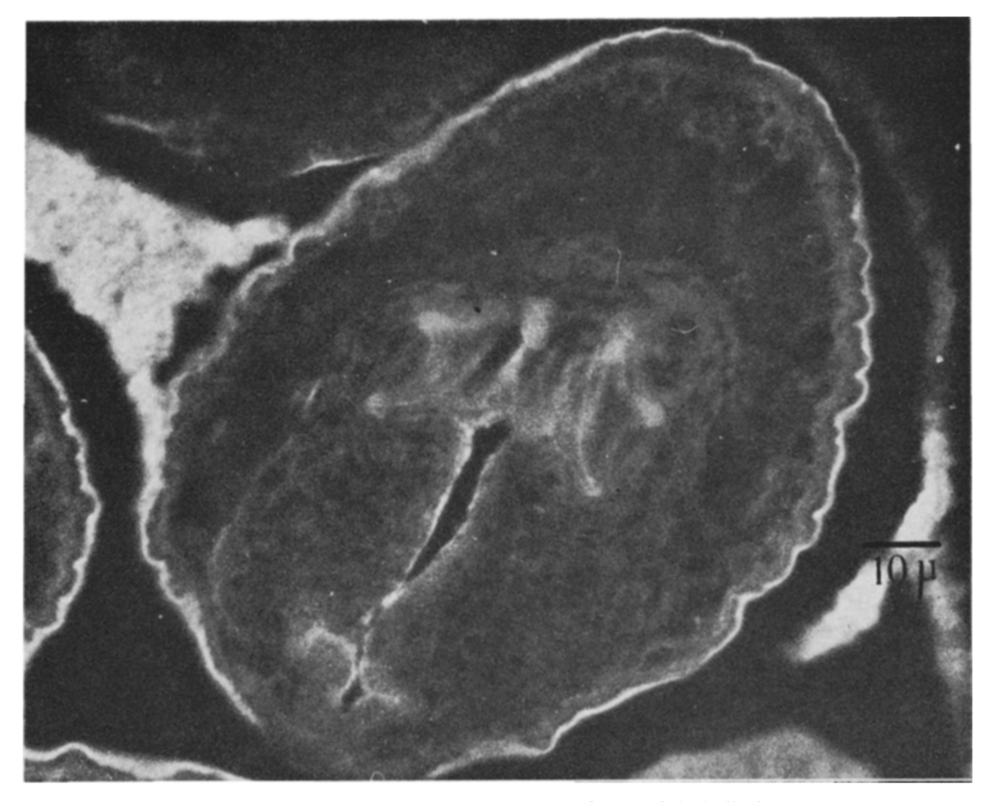


FIG. 4. Section of *Echinococcus granulosus* protoscoleces "labeled" by monospecific anti-antigen B (×1000).

ent from that described for antigen 5 (Yarzabal, Dupas, Bout, and Capron, 1976). The latter antigen was detected in the internal area of the germinal membrane, the parenchyma, and the excretory system of the protoscoleces (Table I). It seems likely that antigen 5 is moved toward the hydatid cyst cavity by the larval

TABLE I

Comparison of the Distribution and Physicochemical Properties of Antigens "5" and "B" from Hydatid Fluid, and PAS-Positive Substances of the Larval Stage of *Echinococcus granulosus*

Tissue distribution	Antigen "5"	Antigen "B"	PAS-positive substances[a]
Laminated "membrane"	−	+	+
Germinal "membrane"	+	−	−
Brood capsule (interstitium)	−	+	+
Protoscoleces			
Tegument	−	+	+
Parenchyma	+	−	−
Excretory system	+	−	−
Thermostability	−	+	?
Sudan black stain	+	+	?
Schiff reaction	−	+	+
Carboxyl esterase activities (α and β)	+	+	?

[a] Data from Kilejian, Schinazi, and Schwabe (1961).

excretory system but that antigen B is released by the protoscoleces into the cystic cavity. The germinal membrane may release antigen B into the host tissues through the hydatid tegumentary structures. The subtegumental cells may participate in this secretory process.

The localization of antigen B corresponds closely to that of the PAS positive substance demonstrated by Kilejian, Schinazi, and Schwabe (1961). The relationship between antigen B, the glycoproteins identified by Kilejian, Sauer, and Schwabe (1962), and these PAS-positive substances remains to be established. However, our results suggest that antigen B may be linked to these glycoproteins.

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